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(54) Title: MONOCLONAL ANTIBODIES WHICH PROMOTE CENTRAL NERVOUS SYSTEM REMYELINATION

(57) Abstract

Monoclonal IgM antibodies which promote central nervous system remyelination when given to a mammal afflicted with a demyelinating disease are disclosed. These antibodies show multi-organ autoreactivity, and recognize both surface and cytoplasmic determinants on glial cells.

MONOCLONAL ANTIBODIES WHICH PROMOTE
CENTRAL NERVOUS SYSTEM REMYELINATION

Background

       Multiple sclerosis (MS) is a chronic, frequently
5   progressive, inflammatory central nervous system (CNS)
disease characterized pathologically by primary
demyelination, usually without initial axonal injury. The
etiology and pathogenesis of MS are unknown. Several
immunological features of MS, and its moderate association
10 with certain major histocompatibility complex alleles, has
prompted the speculation that MS is an immune-mediated
disease (Hafler, D.A. and Weiner, H.L., Immunol. Today,
10:104-107 (1989); Compston, D.A.S., "Genetic
susceptibility to multiple sclerosis," In: McAlpine's
15 Multiple Sclerosis (Matthews, B. ed), pp 301-319, London:
Churchil Livingstone (1991); Olsson, T., Curr. Opin.
Neurol. Neurosurg., 5:195-202 (1992)).

       An autoimmune hypothesis is supported by the
experimental autoimmune (allergic) encephalomyelitis (EAE)
20 model, where injection of certain myelin components into
genetically susceptible animals leads to T cell-mediated
CNS demyelination (Kabat, E.A. et al., J. Exp. Med.,
85:117-129 (1947); Lublin, F.D., Spinger Semin.
Immunopathol., 8:197-208 (1985)). However, specific
25 autoantigens and pathogenic myelin-reactive T cells have
not been definitively identified in the CNS of MS patients,
nor is MS associated with other autoimmune diseases. An
alternative hypothesis, based upon epidemiological data
(Martyn, C., "The epidemiology of multiple sclerosis. In:
30 McAlpine's Multiple Sclerosis, (Matthews, B. ed), pp 3-40,
London: Churchill Livingstone (1991) is that an
environmental factor, perhaps an unidentified virus,
precipitates an inflammatory response in the CNS, which

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leads to either direct or indirect ("bystander") myelin
destruction, potentially with an induced autoimmune
component (Lampert, P.W., Am. J. Path. 91:176-208 (1978)).
This hypothesis is supported by evidence that several
5   naturally occurring viral infections, both in humans (Rice,
G.P.A., Curr. Opin. Neurol. Neurosurg., 5:188-194 (1992))
and animals (Dal Canto, M.C. and Rabinowitz, S.G., Ann.
Neurol., 11:109-127 (1982)), can cause demyelination. One
commonly utilized experimental viral model is induced by
10 Theiler's murine encephalomyelitis virus (TMEV) (Dal Canto,
M.C., and Lipton, H.L., Am. J. Path., 88:497-500 (1977)).
        The limited efficacy of current therapies for MS and
other demyelinating diseases (Goodkin, D.E. et al., Clev.
Clin. J. Med., 59:63-74 (1992)), has stimulated interest in
15 novel therapies to ameliorate these diseases (Martin, R.,
et al., Ann. Rev. Immunol., 10:153-187 (1992); Steinman,
L.,   Adv. Immunol., 49:357-379 (1992); Weiner, H.L., et
al., Science 259:1321-1324 (1993)). However, due to the
apparently complex etiopathogenesis of these diseases,
20 potentially involving both environmental and autoimmune
factors, the need still exists for an effective treatment
of these demyelinating disorders.

Summary of the Invention
        The present invention relates to the promotion, or
25 stimulation, of remyelination of central nervous system
axons in a mammal. Specifically, the present invention
relates to methods of stimulating the remyelination of
central nervous system (CNS) axons using a monoclonal
antibody obtained from a mammal immunized with spinal cord
30 homogenate (SCH) from a normal mammal (i.e., uninfected
with any demyelinating disease). This monoclonal (mAb) is
referred to herein as SCH94.03, and the hybridoma producing
this monoclonal antibody has been deposited on April 28,
1994, under the terms of the Budapest Treaty, with the

American Type Culture Collection (ATCC) and given ATCC
Accession No. CRL 11627. As demonstrated herein, treatment
of a mammal afflicted with a demyelinating disease using
the mAb, SCH94.03, resulted in an increase in CNS
5   remyelination compared to mice treated with control mAb.

The present invention also relates to methods of
treating demyelinating diseases in mammals, such as
multiple sclerosis in humans, and viral diseases of the
central nervous system of humans and domestic animals, such
10   as post-infectious encephalomyelitis, or prophylactly
inhibiting the initiation or progression of demyelination
in these disease states, using the SCH94.03 monoclonal
antibody. This invention further relates to _in vitro_
methods of producing, and stimulating the proliferation
15   of,0 glial cells, such as oligodendrocytes, and the use of
these glial cells to treat demyelinating diseases.

Brief Description of the Figures

Figure 1 is a graph depicting the dose-response
characteristics of antibody-mediated proliferation of cells
20   in mixed rat brain culture.

Figure 2 is a graph depicting the temporal profile of
antibody-mediated proliferation of cells in mixed rat brain
culture.

Figure 3A-3D shows light and electron micrographs of
25   CNS remyelination promoted by mAb SCH94.03. (A) Light
micrograph of spinal cord section from a chronically
infected SJL/J mouse treated with SCH94.03 showing CNS
remyelination. (B) Light micrograph of spinal cord section
from a chronically infected SJL/J mouse treated with a
30   control IgM showing extensive demyelination, and the
relative absence of remyelination. Inflammatory cells,
including macrophages with ingested myelin debris are
indicated by arrows. The asterisk indicates a
representative naked axon. (C) Light micrograph of spinal

cord section with normal myelin. (D) Electron micrograph
of spinal cord section from an animal treated with SCH94.03
showing multiple axons with abnormally thin myelin sheaths
relative to axon diameter. The star in the upper

5   right-hand corner indicates an axon with normal myelin
sheath thickness. Arrowheads point to astrocytic
processes, which are intimately associated with
remyelinated axons. Scale bars represent 13 μm in A-C, and
2 μm in D.

10   Figure 4 is a graph depicting the correlation between
the change in clinical disease and morphological
remyelination.

          Figure 5 is a graph depicting the dose-response
relationship between treatment with mAb SCH94.03 and CNS

15   remyelination. Area of CNS remyelination (●) and
percentage of lesion area with remyelination (○) in animals
treated with various doses of mAb SCH94.03.

          Figure 6 shows a Western blot of TMEV proteins.
Lysates from infected L2 fibroblast cells were separated by

20   SDS-PAGE, transferred to nitrocellulose, and blotted with
SCH94.03 (lane 1), SCH94.32 (lane 2), serum from
susceptible mice chronically infected with TMEV (lane 3),
and polyclonal rabbit anti-TMEV IgG (lane 4). Molecular
weights are indicated on the left in kilodaltons (kDa).

25   The position and identification of the major TMEV capsid
proteins are indicated on the right.

          Figure 7A-7D shows the immunostaining of cultured
glial cells and frozen CNS tissue sections with mAb
SCH94.03. Scale bars represent 15 μm.

30   Figure 8A-8C shows the results of SCH94.03 (Figure 8A)
and control IgMs (Figure 8B and 8C) binding to protein
antigens as determined by ELISA.

          Figure 9 shows the results of SCH94.03 F(ab2)' binding
to protein antigens as determined by ELISA.

    Figure 10A-10C show the results of SCH94.03 (Figure
10A) and control IgMs (Figure 10B and 10C) binding to
chemical haptens as determined by ELISA,

    Figure 11 shows the alignment of the immunoglobulin
5 light and heavy chain variable region sequences of SCH94.03
and control IgM, CH12, and germline Ig gene segments
(SEQ ID NOS:1-11).

Detailed Description of the Invention

    The present invention relates to the promotion, or
10 stimulation, of remyelination of central nervous system
axons in a mammal. Specifically, the present invention
relates to methods of stimulating the remyelination of
central nervous system (CNS) axons using a monoclonal
antibody obtained from a mammal immunized with spinal cord
15 homogenate from a normal mammal (i.e., uninfected with any
demyelinating disease). The antigen reactivity of the
monoclonal antibody, an IgM monoclonal antibody referred to
herein as SCH94.03 (also referred to herein as SCH94.32)
has been characterized as described in the present
20 invention using several biochemical and molecular assays,
including immunohistochemistry, immunocytochemistry,
Western blotting, solid-phase enzyme-linked immunosorbant
assays (ELISA), and Ig variable region sequencing. The
hybridoma producing monoclonal antibody SCH94.03 has been
25 deposited on April 28, 1994, under the terms of the
Budapest Treaty, with the American Type Culture Collection
(ATCC) and has been given ATCC Accession No. CRL 11627.
All restrictions upon the availability of the deposit
material will be irrevocably removed upon granting of the
30 patent.

    The present invention also relates to methods of
treating demyelinating diseases in mammals, such as
multiple sclerosis in humans, and viral diseases of the
central nervous system of humans and domestic animals, such

as post-infectious encephalomyelitis, using the SCH94.03
monoclonal antibody. Methods of prophylactic treatment
using the mAb to inhibit the initiation or progression of
demyelinating diseases are also encompassed by this
5   invention.

Selection of SCH mAbs to promote CNS remyelination
         A panel of monoclonal antibodies (mAbs) derived from
splenocytes of uninfected SJL/J mice injected with SCH was
constructed as described in detail in Example 1. After the
10   initial fusion and cloning, 2 of the 95 wells with viable
Ig-secreting hybridomas contained mAb with significant
binding to SCH as demonstrated by ELISA. Hybridoma cells
from these two wells, called the 79 and 94 series, were
subcloned by limiting dilution and screened again for
15   binding to SCH by ELISA. For the 79 series hybridomas, 14
out of 49 clones were positive by SCH ELISA, while for the
94 series, 17 out of 32 were positive for binding to SCH.
Based upon the ELISA data, two 79 series hybridomas
(SCH79.08 and SCH79.27), both of which also reacted with
20   myelin basic protein (MBP) by ELISA, and three 94 series
hybridomas (SCH94.03, SCH94.11, and SCH94.32), none of
which reacted with MBP, were chosen for ascites production
and in vivo transfer experiments.

MAbs Promote Proliferation of Glial Cells
25   As described in Example 2, the mAbs were tested for
their ability to promote proliferation of glial cells in
vitro. As shown in Table 1, rat optic nerve cells grown in
the presence of mAb 94.02 or 79.27 incorporated more
[^{3}H]thymidine than controls grown in media alone or with an
30   isotype-matched control mAb. Data is shown from one of
five experiments which showed a similar result.

TABLE 1.

Monoclonal Antibodies to Spinal Cord Homogenate Promote Incorporation of [³H]Thymidine in Cultured Optic Nerve Glial Cells

Additive in Medium	µg/ml	CPM Mean + SE	Stimulation Index Isotype Control	P	Stimulation Index PBS	P
mAb 94.32	3	3,642 ± 364	2.68	<0.01	2.17	<0.01
mAb 79.27	3	2,326 ± 182	1.71	<0.01	1.38	<0.05
Isotype Control	3	1,359 ± 82	1.00	--	0.81	--
PBS	--	1,680 ± 203	1.23	--	1.00	--
mAb 94.32	10	4,663 ± 114	2.78	<0.002	1.90	<0.01
mAb 79.27	10	2,711 ± 176	1.62	NS	1.11	NS
Isotype Control	10	1,678 ± 213	1.00	--	0.68	--
PBS	--	2,451 ± 946	1.46	--	1.00	--
mAb 94.32	30	3,855 ± 639	4.01	<0.03	2.44	<0.002
mAb 79.27	30	4,037 ± 371	4.20	<0.04	2.56	<0.003
Isotype Control	30	962 ± 191	1.00	--	0.61	--
PBS	--	1,578 ± 231	1.64	--	1.00	--

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The dose-response characteristics of antibody-mediated
proliferation were then examined. As shown in Figure 1,
maximal stimulation with 94.03 was seen at 100 ng/ml.
Control myeloma IgMs MOPC 104E and TEPC 183 (data not shown)
5 also stimulated the mixed rat brain cultures to proliferate.
However, the maximal effect was seen at a 10-fold higher
concentration than that seen with the mAbs.

The temporal profile of antibody-mediated proliferation
was also examined as shown in Figure 2. On day 8, after
10 culture initiation, 100 ng/ml antibody was added to the
cultures (time 0). Cells were harvested at 24 hour

intervals; [³H]thymidine was present for the final 24 hours
of culture to measure the total proliferation during the
interval. The maximal stimulation with 94.03 was seen at 72
hours after antibody addition. Similar results were
obtained with 94.32. None of the isotype control antibodies
showed any significant proliferation throughout the 120
hours of culture. These data demonstrates that both mAbs
94.32 and 94.03 induce proliferation of glial cells of mixed
rat brain culture. This proliferation is maximal at an
antibody concentration of 100 ng/ml and a culture period of
72 hours after antibody addition.

CNS Remyelination Promoted by mAbs SCH94.03 and SCH94.32

     As described in Example 3, SJL/J mice chronically
infected with TMEV were treated with a total mAb dose of 0.5
mg iv or 5.0 mg ip divided into twice weekly doses for 4-5
weeks. CNS remyelination was measured by a quantitative
morphological assessment on ten spinal cord cross-sections
from each mouse. The criterion for CNS remyelination was
abnormally thin myelin sheaths relative to axonal diameter.
The data are composite of six experiments and are presented
as the mean ± SEM, where n indicates the number of mice.
Statistical comparisons for remyelination data were made
with the cumulative values from both IgM and buffer only
controls using a modified rank sum test. The number of
demyelinated lesions and the area of demyelination were not
significantly different between treatment groups assessed by
a one-way ANOVA. For control IgMs, we used myelomas MOPC
104E and ABPC 22 (both from Sigma), and TB5-1, an anti-
mycobacteria mAb.

     SJL/J mice chronically infected with TMEV and treated
with either mAb SCH94.03 or SCH94.32 showed significantly
greater CNS remyelination than animals treated with either
isotype-matched control mAb or buffer only (Table 2).

Table 2.

Monoclonal antibodies SCH94.03 and SCH94.32 promote CNS remyelination

Treatment	n	Number of Remyelinated Lesions	Area of Remyel (mm²)	p-value	Number of Demyelinated Lesions	Area of Lesion (mm²)	Area Remyelination /Area Lesion (%)
SCH94.03	12	12.8 ± 2.6	0.35 ± 0.09	<0.0025	25.8 ± 2.6	1.09 ± 0.19	28.9 ± 3.8
SCH94.32	12	12.3 ± 2.3	0.42 ± 0.11	<0.0001	24.9 P ± 2.8	1.46 ± 0.21	26.7 ± 4.2
IgM control	13	6.7 ± 1.2	0.11 ± 0.02	--	29.9 ± 2.0	1.70 ± 0.28	7.7 ± 1.8
Buffer only	11	5.1 ± 1.1	0.06 ± 0.01	--	27.7 ± 2.7	1.11 ± 0.29	6.5 ± 1.2

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Remyelination was seen with either iv or ip injections.
SCH94.03- or SCH94.32-treated animals had approximately 2-3-
fold more remyelinated lesions, and a 3-4-fold larger total
area of CNS remyelination than control animals. When a
5 cumulative statistical comparison was made using these two
parameters of therapeutic effectiveness, the CNS
remyelination induced by mAbs SCH94.03 and SCH94.32 was
highly significant ($p < 0.005$; Table 2). In a chronic
progressive disease like TMEV infection, the extent of CNS
10 repair is a direct function of the extent of CNS damage.
Both the number and area of CNS lesions were not different
between treatment groups, indicating similar disease
severity (Table 2). When CNS remyelination was expressed as
the percentage of lesion area showing remyelination,
15 approximately one-third of the cumulative demyelinated
lesion area showed CNS remyelination in mice treated with
either mAb SCH94.03 or SCH94.32 (Table 2).

Morphology of CNS Remyelination

CNS remyelination was readily identified
20 morphologically both by light and electron microscopy
(Figure 3A-3D). Figure 3A shows a remyelinated lesion from
an animal treated with SCH94.03. The majority of axons in
the lesion show morphologic evidence of repair, with
abnormally thin myelin sheaths relative to axonal diameter
25 (Ludwin, S.K. "Remyelination in the central nervous system
of the mouse," In: THE PATHOLOGY OF THE MYELINATED AXON
(Adachi M, Hirano A, Aronson SM eds), pp 49-79, Tokyo:
Igaku-Shoin Ltd. (1985); Ludwin, S.K., Adv. Neurol.,
47:215-254 (1988)). For comparison, Figure 3B shows a
30 demyelinated lesion, with minimal remyelination, whereas
Figure 3C is an area of normal myelin, with thickly
myelinated axons. Within remyelinated lesions (Figure 3A),
there were 15.3 ± 1.0 (mean ± SEM) myelinated axons per 100
μm^2, compared to only 1.1 ± 0.2 myelinated axons per 100 μm^2

in demyelinated lesions (Figure
light micrograph of spinal cord se
By electron microscopy, CNS remyelinat
evident (Figure 3D). Almost every axon
5 evidence of new myelin formation, although myelin.
remyelination (ie. myelin thickness) is variably
individual axons, suggesting different stages of the
process. The ratio of myelin thickness to axonal diame
was 0.08 ± 0.01 (mean ± SEM; n = 25 axons) for remyelinated
10 axons compared to 0.21 ± 0.01 (n = 34 axons) for normally
myelinated axons.

Correlation Between Clinical Disease and Morphological Remyelination

The correlation of morphological remyelination with
15 clinical signs of disease improvement was assessed as
described in Example 3. At each treatment injection, mice
were assessed clinically as described in Example 3. The
change in clinical score was correlated with the percentage
of lesion area showing remyelination (Figure 4).
20 Morphological remyelination is represented as the percentage
of lesion area showing CNS remyelination. A change in
clinical score of 0 represent stable disease over the
treatment period (4-5 weeks), whereas a positive change
indicates worsening of clinical disease, and a negative
25 change indicates improvement. Data represent individual
animals from all treatment groups. A positive change in
clinical score indicates worsening of disease. Using data
from all treatment groups, the change in clinical score
showed a moderate but significant negative correlation
30 (R=-0.40; p < 0.04) with the percentage of lesion area
showing remyelination. Although few animals actually
improved clinically (Δ clinical score < 0), animals with an
increase in disease severity (Δ clinical score > 0) tended
to have less morphological remyelination, while animals that

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remained stable clinically (Δ clinical score = 0) showed the most remyelination. A similar negative correlation was obtained when the other quantitative measures of remyelination were used (the number of remyelinated lesions
5 and the area of remyelination) as shown in Table 2. These data demonstrate that remyelination quantitated by morphology is associated with slowing of clinical disease progression.

Titration of mAb SCH94.03 Dose and CNS Remyelination

10   For the initial treatment experiments, a total mAb dose of 25 mg/kg for iv injections and 250 mg/kg for ip injection was empirically chosen. To assess the dose-response characteristics, and to determine the minimal amount of mAb needed to promote remyelination, chronically-infected mice
15 were treated with various ip doses of SCH94.03. Both the number of remyelinated lesions (data not shown) and the total area of remyelination (Figure 5) increased significantly with larger doses of SCH94.03. Remyelination was quantitated as described for Table 2. Data are the mean
20 values of 4-5 animals per mAb dose, with the final cumulative dose indicated on the graph. SEM averaged 35% of the mean. There was no statistical difference assessed by one-way ANOVA in the number of demyelinated lesions or the area of demyelination between treatment groups, indicating
25 similar extent of disease in all animals. The number of demyelinated lesions and area of lesions were 33.2 ± 7.5 and 1.25 ± 0.43 for the 1000 μg group, 31.8 ± 8 and 1.11 ± 0.31 for the 100 μg group, 23.8 ± 3.4 and 0.54 ± 0.14 for the 10 μg group, and 29.0 ± 6.5 and 0.74 ± 0.20 for the buffer only
30 group (represented as the 0 dose point on the graph). Animals treated with 100 μg control IgM (MOPC 104E) had remyelination scores similar to control animals treated with buffer only. The positive correlation between the dose of mAb SCH94.03 and CNS remyelination was especially striking

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when the severity of CNS disease was taken into account.
When CNS repair was expressed as the percentage of lesion
area showing remyelination, mice treated with a total dose
of 1000, 100, or 10 μg of SCH94.03 had 6-, 5-, and 4-fold
5   more remyelination than control animals, respectively
(Figure 5). Mice given as little as 10 μg of SCH94.03 ip
(0.5 mg/kg) showed evidence of enhanced CNS remyelination.
These data indicated that mAb SCH94.03 and CNS remyelination
had a positive dose-response relationship, and that very
10 small quantities of mAb were needed to promote myelin
repair.

Antigen Specificity of SCH94.03 and SCH94.32.

        Although mAbs SCH94.03 and SCH94.32 were generated from
splenocytes of uninfected mice, and screened against SCH
15 from uninfected mice, it was directly assessed whether
either mAb could react with TMEV capsid proteins or inhibit
viral infectivity in vitro. By Western blotting (Figure 6),
SCH94.03 and SCH94.32 did not react with any TMEV proteins
recognized by either serum from chronically infected mice or
20 polyclonal IgG from rabbits injected with purified TMEV
(Rodriguez, M., et al., Ann. Neurol., 13:426-433 (1983)).
Western blot of lysates from control mock infected L2 cells
showed single bands with the serum from chronically infected
animals and the polyclonal rabbit anti-TMEV IgG at 32 and 43
25 kDa, respectively, but no reactivity with SCH94.03 or
SCH94.32.
        In addition, no significant inhibition of TMEV
infectivity in vitro with up to 5 μg/ml of either SCH94.03
or SCH94.32, was observed under assay conditions where 50%
30 neutralization was observed with a 1:34,000 dilution of
serum from chronically infected animals. These results
indicated that the therapeutic effect of SCH94.03 and
SCH94.32 was not due to direct inhibition of the virus.

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To initially characterize the antigens recognized by mAbs SCH94.03 and SCH94.32, various cell lines derived from glial (rat C6, mouse G26-20, human U373MG and U87MG), neural (human neuroblastoma), fibroblast (mouse L and 3T3),

5 epithelial (human SCC-9 carcinoma), and lymphocytic (mouse CTLL2) origin were stained. Both mAbs stained internal antigens of all cell lines tested, which indicated that certain antigens recognized by these mAbs were not restricted to unique cell types in vitro. Based on the

10 hypothesis that the therapeutic effect of SCH94.03 and SCH94.32 was due to a CNS-specific interaction, the immunostaining of cultured cells by SCH94.03 and SCH94.32 using the rat glial cell line 5.5B8 was further investigated. This immortalized glial cell line has

15 phenotypic characteristics of both ac and astrocytes, with expression of MBP and 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP), and low but detectable expression of glial fibrillary acidic protein (GFAP) and the lipids or proteins recognized by the mAbs A2B5 and O4

20 (Bozyczko, D. et al., Ann. NY Acad. Sci., 605:350-353 (1990)). SCH94.03 and SCH94.32 recognized both a surface and cytoplasmic determinant on 5.5B8 cells. The surface staining was most prominent on small cells which lay on top of a layer of flat, morphologically differentiated cells

25 (Figure 7A). Surface staining was confirmed by flow cytometry on live cells. When the cell membrane was permeabilized by dehydration or brief treatment with a non-ionic detergent to expose internal antigens, the staining pattern was altered considerably (Figure 7B). The

30 cytoplasmic staining was filamentous, with a dense perinuclear network that extended out into the cell processes. This pattern closely resembled the staining pattern of the intermediate filament cytoskeletal protein vimentin. These data indicated that SCH94.03 and SCH94.32

35 recognized antigens that were not restricted to cells

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derived from the nervous system, but that they did recognize
both surface and cytoplasmic determinants on glial cells.

Immunohistochemical staining of frozen mouse, rat, and
human tissue confirmed that SCH94.03 and SCH94.32 were not
5 CNS-specific mAbs, but rather showed multi-organ reactivity.
Both mAbs immunostained all major organs examined, including
the brain, spinal cord, optic nerve, heart, liver, kidney,
stomach, and small intestine and skeletal muscle. However,
not all cells within an organ stained, suggesting in situ
10 cytological specificity. Within the CNS, SCH94.03 and
SCH94.32 stained predominantly blood vessels, ependymal
cells, and stellate-shaped cells with the morphological
features of glial cells, which were enriched in neonatal
cerebellar, periventricular, and brain stem white matter
15 (Figure 7C), and both neonatal and adult optic nerve.
Similar glial cells positive for SCH94.03 and SCH94.32 were
found in autopsied human brain tissue, especially at the
gray-white matter junction (Figure 7D). Identical
immunostaining results were obtained with mAb SCH94.32.
20 Immunostaining with a control IgM (MOPC 104E) was negative
for all samples and tissue structures which immunostained
with SCH94.03 and SCH94.32.

The identification and characterization of an entire
family of autoantibodies, referred to as "natural" or
25 "physiological" autoantibodies, has influenced traditional
views of autoimmunity and self-reactivity. The natural
autoantibodies that have been studied extensively are
typically IgMs, although other isotypes have been
identified, are reactive toward a wide range of antigens,
30 including cytoskeletal proteins, surface proteins, nucleic
acids, phospholipids, bacterial antigens such as
lipopolysaccharides, and various chemical haptens (reviewed
by Avrameas and Ternynck, Mol. Immunol., 30:1133-1142
(1993)). Natural autoantibodies share extensive idiotypic
35 cross-reactivity or "connectivity", which includes

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expression of similar idiotypes, some of which are expressed
by pathogenic autoantibodies, as well as reactivity toward
common idiotypes expressed on other antibodies. Molecular
analysis has shown that natural autoantibodies are typically
5 encoded by unmutated germline immunoglobulin (Ig) genes,
with few if any somatic mutations, and therefore represent a
substantial fraction of the Ig repertoire, especially in
neonatal animals which have not had extensive exogenous
antigen exposure.

10   The function of natural autoantibodies remains
enigmatic. Several hypotheses have been proposed based upon
their biochemical and molecular characteristics. These
include: (1) clearance of senescent or damage tissue, (2)
providing a first line of immunological defense in the lag
15 period between pathogen exposure and an Ag-specific immune
response, (3) masking autoantigeus from a potentially
pathogenic autoimmune response, (4) immunomodulation,
including shaping of the neonatal immune repertoire via an
idiotypic network, and (5) participation in the positive
20 selection of B cells in the bone marrow, similar to the
process proposed for T cells in the thymus.

        The hypothesis that antibodies SCH94.03 and SCH94.32
were natural autoantibodies was tested. To characterize the
antigen reactivities of SCH94.03 and SCH94.32, several
25 biochemical and molecular assays, including
immunohistochemistry and immunocytochemistry, Western
blotting, solid-phase enzyme-linked immunosorbant assays
(ELISA), and Ig variable region sequencing, were used. As
described below, for all biochemical assays, SCH94.03 and
30 SCH94.32 were indistinguishable. In addition, SCH94.03 and
SCH94.32 had identical Ig variable region sequences, which
confirmed that they were the same mAb.

        A potential mechanism whereby SCH94.03 could stimulate
remyelination in the central nervous system would be to
35 stimulate the proliferation and/or differentiation of cells

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involved in myelinogenesis, primarily oligodendrocytes or
their immature precursors. Thus, it was tested whether
SCH94.03 stained the surface of various cells. Using
immortalized cells, it was determined that SCH94.03 stained
5 two glial cells lines, 5.5B8 (Figure 7A) and 20.2E11, but
did not stain the surface of several other glial cells lines
(10.IA3, 20.2A40, C6, G26-20), a neuroblastoma cell line
(B104), two fibroblast lines (L2, Cos-1), or two
myoblastomas (G8, L6). Similar results were obtained with
10 cells isolated from animal tissues and grown in culture.
SCH94.03 stained the surface of oligodendrocytes, but not
astrocytes, microglia, Schwann cells, myoblasts, or
fibroblasts.

The reactivity of SCH94.03 with proteins from glial and
15 lymphoid cell lines, and tissue lysates from brain, liver,
and intestine by Western blotting was also assessed.
SCH94.03 reacted with multiple bands from all cells and
tissues examined, with prominent reactivity toward bands at
50, 95, 120, and >200 kDa. The exact identity of these
20 protein bands has not been determined.

The reactivity of SCH94.03 with several purified
protein self-antigens by solid-phase ELISA was determined.
(Figure 8A-8C). SCH94.03 showed strong reactivity toward the
RBC antigen spectrin, but also showed consistent reactivity
25 toward hemoglobin, actin, tubulin, and vimentin, and
thyroglobulin, although to a lesser qualitative degree than
toward spectrin. No reactivity was observed with myosin,
transferrin, albumin, lysozyme, or myelin basic protein
under our assay conditions. Six other monoclonal or myeloma
30 IgM controls XXMEN-OE5 (Figure 8B), A2B5, MOPC104E, TEPC183,
01, and CH12 (Figure 8C), were also tested, and no
reactivity with any of the antigens tested was observed.

To confirm the monoclonality of SCH94.03, 18 subclones
of SCH94.03 (9 each from SCH94.03 and SCH94.32 parents) were
35 tested for polyreactivity by solid-phase ELISA. All 18

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subclones showed identical reactivity patterns with the
panel of protein antigens as the parent SCH94.03. To
further support the conclusion that the polyreactivity of
SCH94.03 was via its Fab region, we generated F(ab)$_2$'
5 fragments and assessed their reactivity with the protein
antigens by ELISA (Figure 9). SCH94.03 F(ab)$_2$' fragments
showed similar polyreactivity as the whole IgM molecule.

A panel of chemical haptens coupled to bovine serum
albumin (BSA) was constructed and used to assess SCH94.03
10 reactivity by solid-phase ELISA (Figure 10A-10C). SCH94.03
showed strong reactivity toward fluorescein (FL) and
4-hydroxy-3-nitrophenyl acetic acid (NP), moderate
reactivity toward phenyloxazolone (PhOx), and weak
reactivity toward 2, 4, 6-trinitrophenyl (TNP) and
15 *p*-azophenylarsonic acid (Ars). No reactivity with *p*-
azophenyltrimethylammonium (TMA),
p-azophenylphosphorylcholine (PC), or the carrier protein
BSA was detected. Control IgMs (Figure 10B and 10C) showed
no significant binding to any of the haptens tested, with
20 the exceptions of CH12 reactivity with TMA, which has been
previously reported, and A2B5 reactivity with NP.

It was further investigated whether the Ig light (L)
(SEQ ID NOS:1 and 2) and heavy (H) (SEQ ID NOS:6 and 7)
chains of SCH94.03 were encoded by germline Ig genes (Figure
25 11). The light chain variable (V$_L$) and joining (J$_L$) region
nucleotide sequences from SCH94.03 (SEQ ID NOS:1 and 2) had
99.4% identity with the previously published sequences of
the germline Vκ10 (SEQ ID NO:4) and Jκ1 (SEQ ID NO:5) genes,
with only two silent changes at the 3' end of both the V$_L$
30 and J$_L$ regions. The SCH94.03 V$_H$ (SEQ ID NOS:6 and 7) region
nucleotide sequence was identical to the previously
published germline V$_H$23 (SEQ ID NO:10) sequence, the J$_H$
region sequence differed from the published germline J$_H$2
(SEQ ID NO:11) sequence by one nucleotide, at the 5' end of
35 the J region, and the diversity (D) region contained 15

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contiguous nucleotides derived from the germline DFL16.1
gene. There were 8 nucleotides in the V-D junction, and 1
in the D-J junction, which did not correspond to any known
germline V or D region genes, and probably represent non-
5 coded (N) nucleotides inserted by the enzyme terminal
deoxynucleotide transferase during V-D-J recombination. The
only changes from the germline genes in the heavy chain of
SCH94.03 occurred at either the V-D or D-J junction, and
therefore could represent either N nucleotides or the result
10 of imprecise joining, rather than somatic mutations. In
addition, both the light and heavy chain variable regions of
SCH94.03 showed extensive sequence similarity with the IgM
produced by the B-cell lymphoma CH12 (SEQ ID NOS:3, 8 and 9)
(Figure 11).

15 SCH94.03 is a Natural Autoantibody

These preliminary antigen reactivity results suggest
that SCH94.03 is a natural autoantibody. Although this
conclusion does not readily present a mechanism as to how
SCH94.03 stimulates remyelination in the central nervous
20 system, it does suggest an important physiological function
of natural autoantibodies. Autoantibodies that are produced
either during normal physiology, or in response to tissue
damage and the subsequent release of previously sequestered
antigens, might actively participate to promote repair in
25 the damaged tissue. In line with previously proposed
functions of natural autoantibodies, this active
participation might be to facilitate removal of damaged
tissue, mask autoantigens thereby preventing a vigorous
pathogenic autoimmune response, modulate the immune response
30 which actually resulted in the tissue destruction, thereby
allowing normal endogenous tissue repair to occur, or
directly stimulate cells involved in the repair process.

Thus, as a result of the work described herein, it is
now demonstrated that an autoantibody generated and screened

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for its autoantigen-binding capability, also promotes CNS
remyelination. Mice chronically infected with TMEV and
treated either iv or ip with IgM mAbs from hybridomas
SCH94.03 or SCH94.32 had significantly more CNS repair than

5 control animals, measured by a detailed quantitative
morphological assessment of CNS remyelination. Moreover,
preliminary data suggest that the autoantibody, SCH94.03 is
also effective in promoting remyelination in mammals
afflicted with experimental autoimmune encephalomyelitis

10 (EAE). Thus, it is reasonable to predict that
autoantibodies, such as SCH94.03, play a critical role in
stopping an immune-mediated process of demyelination in CNS
diseases.

      Two potential mechanisms can be proposed by which Abs
15 promote remyelination. First, Abs might inhibit some
pathogenic component of the disease process, such as virus
activity, an immune response which directly induces
demyelination, or an immune response which prevents
remyelination. If the disease outcome is based upon a
20 balance between tissue destruction and repair, inhibition of
pathogenic components would allow a physiological repair
response to predominate. Experimental and clinical evidence
support this hypothesis. Spontaneous CNS remyelination is
seen in MS patients and several experimental models of CNS
25 demyelination as well as described herein, demonstrating
spontaneous remyelination in control mice. This indicates
that remyelination is a normal physiological response to
myelin damage. In addition, treatment of mice chronically
infected with TMEV with various immunosuppressive regiments
30 promotes remyelination, but does not decrease demyelination,
indicating that there is an immunological component which
inhibits remyelination. (Rodriguez, M. and Lindsley, M.D.,
Neurology, 42:348-357 (1992)). Preliminary immunological
function studies have indicated that animals treated with
35 SCH94.03 had similar numbers of B and T (both CD4+ and CD8+)

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cells in their spleens compared to control animals, had
similar in vitro splenocyte proliferative responses to
mitogens and antigens, and mounted comparable Ab responses
to both T cell-dependent and T cell-independent antigens.

5   The second hypothesis is that certain Abs can actively
stimulate CNS remyelination, perhaps via stimulation of
oligodendrocyte proliferation and/or differentiation in
vivo, as has been demonstrated in vitro (Diaz, M. et al.,
Brain Res., 154:231-239 (1978); Raine, C.S., et al., Lab.
10 Invest., 38:397-403 (1979); Lehrer, G.M. et al., Brain
Res., 172:557-560 (1979); Bansal, R. et al., J. Neurosci.
Res., 21:260-267 (1988); Benjamins, J.A. and Dyer, C.A.,
Ann. NY Acad. Sci., 605:90-100 (1990); Dyer, C.A., Mol.
Neurobiol., 7:1-22 (1993)). MAb SCH94.03 may directly
15 stimulate precursor glial cells which are known to be
present at the edges of both human and experimental CNS
lesions which show active remyelination. Alternatively,
SCH94.03 may work indirectly, via activation of astrocytes
or other accessory cells, which could release factors
20 important for the survival or proliferation of cells in the
oligodendroglial lineage. The formation of Ab-antigen
complexes in situ with tissue components released upon
myelin destruction may also participate in Ab-mediated CNS
remyelination. Although SCH94.03 is not CNS-specific, the
25 recognition of both surface and cytoplasmic antigens on
glial cells by the mAb supports an active mechanism
hypothesis. In contrast to the immunomodulatory hypothesis,
which would not necessarily require that Abs have direct
access to the CNS, the hypothesis that Abs actively
30 stimulate CNS remyelination implies the prerequisite of
direct access to the CNS. This is contrary to the view of
the selective permeability of the blood-brain barrier,
especially toward large molecules such as pentameric IgM.
However, during chronic inflammatory conditions such as TMEV
35 infection or MS, peripheral leukocytes migrate into the CNS,

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indicating an alteration in the blood-brain barrier
permeability. Therefore, large proteins such as serum Ig
might also enter, via either passive diffusion through
"open" endothelium, or perhaps via an unidentified active
5 transport mechanism.

Treatment of Demyelinating Diseases

     The results of the experiments described herein have
practical applications to multiple sclerosis (MS), EAE, and
other related central nervous system demyelinating
10 disorders. Rare examples of spontaneous CNS-type
remyelination ("shadow plaques") are found in MS and
occasional peripheral nervous system (PNS)-type
remyelination is found in demyelinated spinal cord plaques
near the root entry zone. Oligodendrocytes are infrequent
15 at the center of the chronic plaques in MS but they appear
to proliferate at the periphery of plaques, where they are
associated with abortive remyelination. The process of
remyelination may correlate with the spontaneous remission
and improvements observed clinically in MS. These clinical
20 observations indicate that new myelin formation is possible
in MS. The remyelination that has been stimulated in mice
with TMEV-induced demyelination by using a mAb may hold
promise for therapeutic application in multiple sclerosis.
     Of importance clinically is the question of whether
25 morphologic regeneration of thin myelin sheaths contributes
to functional recovery. Computer simulations indicate that
new myelin formation even by inappropriately thin sheaths
improves impulse conduction. Since the axon membrane of
normally myelinated fibers is highly differentiated, it is
30 necessary for sodium channels to be present at high density
at the node of Ranvier to propagate saltatory conduction.
Experimental evidence suggests that newly formed nodes do
develop the required high sodium channel density as
demonstrated by saxitoxin binding. Data to date suggest

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that remyelination even by inappropriately thin myelin
improves conduction in a previously demyelinated axon.
Therefore, any strategy to promote this morphologic
phenomenon has the potential of producing functional
5 recovery.

The data presented herein demonstrates, for the first
time, that administration of a monoclonal antibody to a
mammal is capable of stimulating remyelination of central
nervous system axons _in_ _vivo_. Specifically, treatment of
10 chronically infected TMEV-infected mice with as little as 10
ug of SCH94.03 resulted in a 4- to 5-fold increase in the
total area of CNS myelination compared to mice treated with
a control mAb.

Thus, as a result of the experiments described herein,
15 the method of the present invention can be used to treat
mammals, including humans and domestic animals, afflicted
with demyelinating disorders, and to stimulate remyelination
of the CNS axons. As described herein, an effective amount
of the monoclonal antibody can be administered by
20 intravenous (iv) or intraperitoneal (ip) injection. An
effective amount of the antibody can vary depending on the
size of the mammal being treated, the severity of the
disease, the route of administration, and the course of
treatment. For example, each dose of mAb administered can
25 range from approximately 0.5 mg/kg to approximately 400
mg/kg, with the preferred range from approximately 0.5 mg/kg
to approximately 250 mg/kg. It is important to note that a
dose as low as 10 μg (0.5 mg/kg) was effective in promoting
remyelination of CNS axons in mice. The dose of mAb will
30 also depend on the route of administration. For example, an
iv dose administered to mice was 0.5 mg/kg, and an ip dose
was 5.0 mg/kg. The course of treatment includes the
frequency of administration of the mAb (e.g, daily, weekly,
or bi-weekly) and the duration of the treatment (e.g, four
35 weeks to four months). Thus, for example, a larger amount

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of mAb can be given daily for four to five weeks, as opposed·
to a smaller amount of mAb given for four months.

The effectiveness of the amount of the monoclonal
antibody being administered can be assessed using any number
5 of clinical criteria, for example, as described in Example
3, including overall appearance of the mammal, the activity
of the mammal and the extent of paralysis of the mammal.
The effectiveness of the amount of monoclonal antibody
necessary to induce remyelination in humans can also be
10 assessed in a double blinded controlled trial. Patients
with fixed neurological deficits from demyelinating disease
can be treated with monoclonal antibody or controls.
Improvement in isometric muscle strength as detected by
quantitative biomechanics muscle testing could be used as
15 the primary therapeutic end-point.

An effective amount of the monoclonal antibody can be
combined with, or diluted with, an appropriate
pharmaceutically acceptable carrier, such as a physiological
buffer, or saline solution. Additionally, the monoclonal
20 antibody may be genetically altered, e.g. "humanized" by the
substitution of human antibody nucleotide sequences in non-
variable regions of the murine mAb to reduce immunogenicity.

In addition to in vivo methods of promoting
remyelination, ex vivo methods of stimulating remyelination
25 in CNS axons are also encompassed by the present invention.
For example, the monoclonal antibody may be used in vitro to
stimulate the proliferation and/or differentiation of glial
cells, such as oligodendrocytes, as described in Example 2.
These exogenous glial cells can then be introduced into the
30 CNS of mammals using known techniques. Remyelination of CNS
axons would be increased by increasing the number of
endogenous glial cells present (glial cells, such as
oligodendrocytes play a critical role in the production of
myelin).

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In _vitro_ methods of producing glial cells, or
stimulating the proliferation of glial cells from mixed
culture (e.g., rat optic nerve cell, or rat brain cell
cultures) are also encompassed by this invention. For
5 example, cells obtained from rat optic nerve, or rat brain,
containing glial cells, are cultured as a mixed culture
under conditions sufficient to promote growth of the cells.
An effective amount of mAb capable of promoting
remyelination of CNS axons, such as SCH94.03, is then added
10 to the mixed culture of cells and maintained under
conditions sufficient for growth and proliferation of cells.
The mAb stimulates the proliferation of glial cells in the
mixed culture. Thus the proliferation of glial cells
cultured in the presence of the mAb is increased, relative
15 to the proliferation of glial cells grown in the absence of
the mAb.

The invention will be further and more specifically
illustrated by the following Examples, which are not
intended to be limiting in any way.

20 Example 1: Monoclonal Antibody Production, Screening and
Purification

Animals

Spleens of two SJL/J mice (Jackson Laboratories, Bar
Harbor, ME) that had been injected twice with spinal cord
25 homogenate (SCH) in incomplete Freund's adjuvant were used
as the source of B cells for fusion and hybridoma
production. Splenocytes were fused with NS-1 myeloma cells
using polyethylene glycol, and viable cell fusions were
selected with hypoxanthine-aminopterin-thymidine (HAT) media
30 and cloned by limiting dilution as described (Katzmann, J.A.
et al., _Proc. Nat. Acad. Sci. USA_, 78:162-166 (1981)).

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ELISAs

Hybridoma supernatants from viable Ig-producing clones
were screened for binding to SCH by an enzyme-linked
immunosorbant assay (ELISA). The following antigens were
5 used for screening mAbs: SCH - (10 µg) reconstituted in
carbonate-bicarbonate buffer (pH 8.53), MBP - (1 µg)
dissolved in PBS, GC (1 µg) dissolved in absolute alcohol,
PLP (1 µg) dissolved in water. PLP was provided by Dr. W.
Macklin (UCLA) who has published a solid phase immunoassay
10 for PLP. For SCH, MBP or GC ELISA, Immuno II plates were
coated with prepared antigen (100 µl/well) which was
incubated overnight at 4°C. The following day wells were
washed in PBS and blocked with PBS + 1% serum for 1 hr at
room temperature. Plates were washed again in PBS and
15 serial dilutions of primary Ab diluted in PBS/0.1% BSA were
added and incubated at room temperature for 2 hrs. Plates
were washed in PBS/0.05% Tween and appropriate secondary Ab
conjugated to alkaline phosphatase (1:1000 in PBS 0.1% BSA)
was added. Plates were incubated at 37°C for 2 hrs, washed
20 in PBS 0.05% Tween, and the substrate (Sigma 104 Phosphatase
Substrate Tablet in 5 ml diethanolamine buffer) was added
for 30 min. The reaction was terminated with 50 µl of 1 N
NaOH. The plates were read on a Dynatech ELISA plate
reader.

25 Ascites production

The hybridomas chosen for treatment experiments were
injected into pristane-treated BALB/c mice for ascites
production. Hylridomas were also grown in RPM1-1640 media
supplemented with 10% fetal bovine serum for IgM production.
30 IgM mAbs were purified by either ammonium sulfate
precipitation and gel filtration on a Sephacryl S-400 HR
(Sigma) column for the initial transfer experiments, or by
affinity chromatography using goat anti-mouse IgM (µ-chain
specific; Jackson Immunoresearch, West Grove, PA) coupled to

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Reacti-Gel 6X matrix (Pierce, Rockford, IL) for later
transfer experiments.

Example 2: In Vitro Testing of Monoclonal Antibodies

Selection of mAbs that promote glial cell proliferation

5   The ability of the mAbs to promote proliferation of
glial cells in vitro was tested. Glial cells isolated from
rat brain or optic nerves were seeded in Falcon Microtest II
plates at a concentration of 2 x 10⁴ cells per well in 0.1
ml of DME. Whole serum (SCH, IFA, MBP, GC, MBP/GC, PBS or
10 PLP), purified Ig or mAb, was serially diluted and 0.1 ml
aliquot was added to cells and assayed in triplicate. Three
days later ³H-thymidine was added (1 μCi/ml) and cells were
harvested after 17 hrs with an automated cell harvester
(Mash II Harvester). To document identity of cells
15 proliferating (i.e., , astrocytes, progenitor glial cells,
macrophages), selected cultures after exposure to
³H-thymidine, were incubated with appropriate Ab specific
for cell type followed by ABC immunoperoxidase technique.
After reaction of Hanker-Yates reagent, the slides were
20 immersed in Ilford K2 nuclear emulsions, exposed for 4 days
at 4°C and developed.

mAb 94.03 and 94.32 induce proliferation of mixed rat optic
nerve brain cultures

       One- to two-day-old rats were killed with ether.
Through careful dissection, optic nerves were removed from
5 the optic nerve chiasm to the eye. Nerves were transferred
to centrifuge tubes containing 2 mls of DMEM. An equal
volume of 0.25% trypsin was added and incubated to 37°C in a
water bath for 45 min. 0.2 ml of FCS was added to terminate
trypsinization. Nerves were passed through a sterile needle
10 and syringe (gauge no. 21) and then centrifuged at 1400 rpm
for 10 min. The cell count was adjusted to provide

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concentration of 5 x 10^5 cells/100 μl of media in 24-well
trays in DMEM + 0.5% FCS. After 12 to 16 hrs, appropriate
antibodies or growth media were added as per experimental
protocols.

5   Brains of 1-2 day old rats were removed and placed in
Hank's Balanced Salt Solution with 10 mM HEPES buffer
(HBSS/H), approximately 1-2 ml per brain. The brain stem,
cerebellum, and midbrain was discarded whereas the forebrain
was minced with a bent syringe. The tissue was further
10 disrupted by repeated passage through a 10 ml pipet and
transferred to a 50 ml conical tube. The tissue suspension
was shaken on a rotary shaker (75 rpm) for 30 min at 37°C.
Trypsin was added to a final concentration of 0.125% and the
suspension was shaken for an additional 60 min. Trypsin
15 digestion was stopped by adding FCS (10%). The cell
suspension was passed sequentially through 120 and 54 μm
Nytex, centrifuged, resuspended in serum-free medium with
10% FCS, and filtered again through 54 μm Nytex. Serum-free
media was DMEM with 3.7 g/l sodium bicarbonate, 6.0 g/l
20 glucose, 2 mM L-glutamine, 0.1 nM nonessential amino acids,
5 μg/ml insulin, 5 μg/ml transferrin, 5 ng/ml selenite, 100
U/ml penicillin and 100 μg/ml streptomycin. The cells were
counted, plated onto uncoated tissue culture flasks or
plates at 5x10^4 cells/cm^2 and cultured at 37°C in 5% CO_2.
25 The media was changed after 72 hrs, and every 48 hrs
thereafter. On day 8 after culture initiation, the media
was aspirated and replaced by SFM with various supplements
(for example, antibody). For most experiments, the cells
were grown for an additional 48 hrs before harvesting.
30 Cells were pulsed with [^{3}H]thymidine (5 μCi/ml) for the
final 1824 hrs of culture.

Western Blot Procedure

Antigens were denatured and solubilized by heating at
100°C in sodium dodecyl sulfate (SDS) sample buffer.

Samples were electrophoresed on stacking and separating gels
containing 4.75% and 12.0% acrylamide at 200 volts. After
electrophoresis, gels and nitrocellulose membranes were
equilibrated for 30 min in transfer buffer (25 mM Tris, 192
5 mM glycine, 20% methanol, pH 8.1-8.3). All steps were done
at room temperature. Gels were electroblotted for either 1
hr at 100V or overnight at 30V using the Bio-Rad Mini
Trans-blot apparatus. The nitrocellulose membrane was cut
into strips and washed, 3X TBS (100 mM NaCI, 50 mM TriG, pH
10 7.6) with 0.03% Tween 20. Nitrocellulose strips were
blocked (TBS with 3% non-fat milk and 0.03% Tween 20) for
2-4 hrs, washed 3X, and incubated with primary Ab or
antisera (diluted in blocking buffer) for 4 hrs or
overnight. After primary Ab incubation, strips were washed
15 3X, incubated with either biotin- or alkaline
phosphate-labelled secondary Ab (diluted in blocking buffer)
for 2 hrs, washed 3X, and incubated with
alkaline-phosphatase labeled-streptavidin (diluted in
blocking buffer) for 2 hrs if the biotin system is used.
20 Nitrocellulose strips were washed 4X (final wash in TBS
without Tween 20) and incubated with substrate solution
(0.165 mg/ml BCIP and 0.33 mg/ml NBT in 100 mM NaCI, 100 mM
TriG, 5 mM MgG12, pH 9.5) until sufficient color developed
(approximately 10-15 min). The reaction was stopped by
25 adding PBS with 5 mM EDTA.

Cell lines or mixed brain cultures were lysed in 1X SDS
reducing sample buffer (2.3% SDS, 10% 2-ME, 0.125 M Tris,
20% glycerol) and heated to 85°C for 15 min. Nucleic acids
were sheared by repeated passage of lysate through 21-
30 27-gauge needles. Lysate proteins were separated on a 12%
acrylamide reducing gel, transferred to nitrocellulose
membranes, and blotted with various antibodies as previously
described.

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Example 3: Promotion of CNS Remyelination Using a Monoclonal Antibody

Virus

        The DA strain of TMEV was obtained from Drs. J. Lehrich
5 and B. Arnason after eight passages in BHK cells. The virus
    was passaged an additional four times at a multiplicity of
    infection of 0.1 plaque forming units (PFU) per cell.
    Cell-associated virus was released by freeze-thawing the
    cultures followed by sonication. The lysate was clarified
10 by centrifugation and stored in aliquots at -70°C. All
    subsequent experiments will use passage 12 virus. This
    virus isolate causes white matter pathology without
    destruction of anterior horn cells.

In vitro TMEV neutralization assay
15   Viral plaque assays were done as previously described
    (Patick, A.K., et al., J. Neuropath. Exp. Neurol.,
    50:523-537 (1991)). To assess neutralization, aliquots of
    TMEV (200 PFU/ml) were incubated with various concentrations
    of Ab for 1 hour at room temperature prior to plating onto
20 confluent L2 cells. As a positive control, we used serum
    from susceptible mice chronically infected with TMEV. Under
    the assay conditions described above, a serum dilution of
    1:34,000 gave 50% neutralization, which corresponded to an
    estimated 20 ng/ml of TMEV-specific Abs, assuming a total
25 serum Ig concentration of 15 mg/ml, and a TMEV-specific
    fraction of 5%.

Demyelination protocol
        Demyelination was induced in female SJL/J mice, ages
    four to six weeks, from the Jackson Laboratory, Bar Harbor,
30 ME. Mice were inoculated intracerebrally with 2×10^5
    plaque-forming units of DA virus in a volume of 10 μl. Mice

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infected chronically with TMEV (4 to 6 months following
infection) were assigned randomly to groups of treatment.

Treatment protocol and clinical disease assessment

5   Chronically infected mice were given either intraperitoneal
    (ip) or intravenous (iv) injections of mAb twice weekly for
    4-5 weeks. At each treatment injection, mice were assessed
    clinically by three criteria: appearance, activity, and
    paralysis. A score for each criterion was given ranging
10 from 0 (no disease) to 3 (severe disease). For appearance,
    1 indicated minimal change in coat, 2 indicated a moderate
    change (scruffy appearance), and 3 indicated a severe change
    (incontinence and stained coat). For activity, 1 indicated
    decreased spontaneous movements (minimal ataxia), 2
15 indicated moderate slowing (minimal spontaneous movements),
    and 3 indicated severe slowing (no spontaneous movement).
    For paralysis, 0.5 indicated a spastic extremity, 1
    indicated a paralyzed extremity, 1.5 indicated two or more
    spastic extremities, 2 indicated two paralyzed extremities
20 (unable to walk), 2.5 indicated no righting response, and 3
    indicated three or four paralyzed extremities (moribund).
    The total score for each mouse was the cumulative total from
    each criterion (maximum of 9). As the clinical score was an
    ordinal, but not a cardinal scale, the change in clinical
25 score to assess clinical disease was used. The clinical
    assessment data were not disclosed until after the
    morphological assessment of remyelination was completed.

Light and electron micrograph preparation and assessment of
remyelination

30   Preparation of light and electron microscopy sections
    and morphological assessment of remyelination were done.
    Briefly, treated mice were anesthetized with pentobarbital
    (0.2 mg ip), exsanguinated by cardiac puncture, and killed
    by intracardiac perfusion with Trump's fixative (100 mM

phosphate buffer, pH 7.2, with 4% formaldehyde and 1.5%
glutaraldehyde). The entire spinal cord was removed
carefully from the spinal canal, and sectioned into 1 mm
transverse blocks. Every third block was post-fixed in 1%
5 osmium tetroxide and embedded in Araldite (Polysciences,
Warrington, PA). One micron sections from each block were
cut and stained with p-phenylenediamine. On each section,
remyelination was quantitated using a Zeiss interactive
digital analysis system (ZIDAS) and camera lucida attached
10 to a Zeiss photomicroscope (Carl Zeiss Inc., Thornwood, NY).
Abnormally thin myelin sheaths relative to axonal diameter
was used as the criterion for CNS remyelination. Ten spinal
cord sections from each mouse were examined; this
corresponded to 8-9 mm^2 of white matter examined per mouse.
15 To avoid bias, slides were coded and quantitation was done
without knowledge of the treatment groups.

Myelin thickness and axonal diameter measurements and
quantitation of myelinated axons

        Electron micrographs of normal and remyelinated axons
20 from plastic-embedded spinal cord sections were imaged with
a Hamamatsu video camera, digitized, and analyzed using an
IBAS 2000 Image Analysis System (Kontron, Munich, Germany).
The axonal cross-sectional area with and without the myelin
sheath was measured, and equivalent circle calculations were
25 used to determine the axonal diameter and myelin sheath
thickness. For myelinated axon quantitation, the number of
myelinated axons in lesions from plastic-embedded spinal
cord sections were counted using the analysis system
described above attached to an Axiophot microscope (Carl
30 Zeiss, Inc.). 17 remyelinated and 15 demyelinated lesions
in spinal cord sections from animals treated with mAb
SCH94.03, control IgM, or buffer only were analyzed. This
corresponded to 0.6 mm^2 of remyelinated area and 0.8 mm^2 of
demyelinated area. The criterion for selection of a lesion

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as demyelinated was the presence of substantial
demyelination with minimal repair, whereas remyelinated
lesions were chosen based upon the presence of almost
complete remyelination throughout the lesion.

5 <u>Immunostaining</u>

Rat 5.5B8 glial cells were grown on
poly-D/L-lysine-coated chamber slides in Dulbecco's modified
Eagle's medium (DMEM) supplemented with 1.5 g/L D-glucose,
30 nM SeO_2, 15 nM triiodothyronine, 10 ng/ml biotin, 100 μM
10 $ZnCl_2$, 50 μg/ml gentamicin, and 10% fetal bovine serum. All
staining steps were done at room temperature. For surface
staining, slides were briefly rinsed with PBS, and cells
were lightly fixed with 1% formaldehyde in PBS for 10 min to
prevent cell detachment during subsequent staining steps.
15 For cytoplasmic staining, slides were rinsed twice in PBS
and either air dried for 1 hour or incubated with 0.1%
Triton X-100 in PBS for 10 min. Cells were blocked in 2%
BSA for 30 min, washed, incubated with control IgM or mAb
SCH94.03 (10 μg/ml in 1% BSA) for 1 hour, and washed
20 extensively with PBS. After fixation with 4%
paraformaldehyde for 15 min, slides were incubated with
fluorescein-labeled goat anti-mouse IgM (Jackson
Immunoresearch) for 1 hour, washed with PBS, coverslipped
with 10% MOWIOL® (Hoechst) in 100 mM Tris, 25% glycerol, pH
25 8.5 with 25 μg/ml 1,4-diazobicyclo-[2.2.2]-octane (DABCO) to
prevent fading, and allowed to set overnight in the dark.
For frozen tissue sections, fresh neonatal rat, adult mouse,
or autopsied human cortical brain tissue was quick frozen in
isopentane chilled in liquid nitrogen prior to liquid
30 nitrogen storage. Frozen sections (10 μm) were transferred
onto gelatinized glass microscope slides, air dried for 4-8
hours, and stored at -70°C. Prior to immunostaining, slides
were placed at room temperature overnight. The
immunoperoxidase staining protocol was similar to that

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described above, using the ABC immunoperoxidase reagent
(Vector Laboratories, Burlingame, CA), developed with 1.5
mg/ml Hanker-Yates reagent (p-phenylene diamine-procatechol)
in 50 mM Tris, pH 7.6 with 0.034% H_2O_2, counterstained with
5 Mayer's hematoxylin, and mounted with Permount (Fischer
Scientific, Pittsburgh, PA).

Data Analysis

A modified cumulative rank sum test (O'Brien, P.C.,
Biometrics, 40:1079-1087 (1984)) was used to compare
10 remyelination between treatment groups. This statistical
test takes into account several numerically unrelated
parameters of therapeutic effectiveness, and is used
routinely for clinical trial efficacy assessment. Parallel
analyses using a standard unpaired Student's t-test to
15 compare individual parameters of remyelination gave
equivalent results. Comparisons of disease severity and
correlation significance were determined by a one-way
analysis of variance (ANOVA). Statistical analyses were
done with the either the SigmaStat (Jandel Scientific, San
20 Rafael, CA) or EXCEL (Microsoft Corporation, Redmond, WA)
software programs. Calculated values were considered
significant when p was < 0.05.

Equivalents

Those skilled in the art will recognize, or be able to
25 ascertain, using no more than routine experimentation, many
equivalents to the specific embodiments of the invention
described herein. Such equivalents are intended to be
encompassed by the following claims:

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

   (i) APPLICANT: Mayo Foundation for Medical Education Research

   (ii) TITLE OF INVENTION: MONOCLONAL ANTIBODIES WHICH PROMOTE
        CENTRAL NERVOUS SYSTEM REMYELINATION

  (iii) NUMBER OF SEQUENCES: 11

   (iv) CORRESPONDENCE ADDRESS:
        (A) ADDRESSEE: Hamilton, Brook, Smithy & Reynolds, P.C.
        (B) STREET: Two Militia Drive
        (C) CITY: Lexington
        (D) STATE: Massachusetts
        (E) COUNTRY: USA
        (F) ZIP: 02173

    (v) COMPUTER READABLE FORM:
        (A) MEDIUM TYPE: Floppy disk
        (B) COMPUTER: IBM PC compatible
        (C) OPERATING SYSTEM: PC-DOS/MS-DOS
        (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

   (vi) CURRENT APPLICATION DATA:
        (A) APPLICATION NUMBER:
        (B) FILING DATE:
        (C) CLASSIFICATION:

  (vii) PRIOR APPLICATION DATA:
        (A) APPLICATION NUMBER: U.S. 08/236,520
        (B) FILING DATE: April 29, 1994

 (viii) ATTORNEY/AGENT INFORMATION:
        (A) NAME: Granahan, Patricia
        (B) REGISTRATION NUMBER: 27,227
        (C) REFERENCE/DOCKET NUMBER: MMV92-01 PCT

   (ix) TELECOMMUNICATION INFORMATION:
        (A) TELEPHONE: 617-861-6240
        (B) TELEFAX: 617-861-9540

(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 393 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

   (ix) FEATURE:
        (A) NAME/KEY: CDS
        (B) LOCATION: 1..393

-36-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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ATG ATG TCC TCT GCT CAG TTC CTT GGT CTC CTG TTG CTC TGT TTT CAA       48
Met Met Ser Ser Ala Gln Phe Leu Gly Leu Leu Leu Leu Cys Phe Gln
1               5                   10                  15

GGT ACC AGA TGT GAT ATC CAG ATG ACA CAG ACT ACA TCC TCC CTG TCT       96
Gly Thr Arg Cys Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser
                20                  25                  30

GCC TCT CTG GGA GAC AGA GTC ACC ATC AGT TGC AGG GCA AGT CAG GAC      144
Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp
        35                  40                  45

ATT AGC AAT TAT TTA AAC TGG TAT CAG CAG AAA CCA GAT GGA ACT GTT      192
Ile Ser Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val
    50                  55                  60

AAA CTC CTG ATC TAC TAC ACA TCA AGA TTA CAC TCA GGA GTC CCA TCA      240
Lys Leu Leu Ile Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser
65                  70                  75                  80

AGG TTC AGT GGC AGT GGG TCT GGA ACA GAT TAT TCT CTC ACC ATT AGC      288
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser
                85                  90                  95

AAC CTG GAG CAA GAA GAT ATT GCC ACT TAC TTT TGC CAA CAG GGT AAT      336
Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn
                100                 105                 110

ACG CTT CCG TGG ACG TTC GGT GGA GGC ACC AAG CTG GAA ATC AAA CGG      384
Thr Leu Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
            115                 120                 125

GCT GAT GCT                                                          393
Ala Asp Ala
        130
```

(2) INFORMATION FOR SEQ ID NO:2:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 131 amino acids
          (B) TYPE: amino acid
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: protein

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Met Ser Ser Ala Gln Phe Leu Gly Leu Leu Leu Leu Cys Phe Gln
1               5                   10                  15

Gly Thr Arg Cys Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser
                20                  25                  30

Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp
        35                  40                  45

Ile Ser Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val
    50                  55                  60
```

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```
Lys Leu Leu Ile Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser
65               70              75              80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser
                85              90              95

Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn
            100             105             110

Thr Leu Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
            115             120             125

Ala Asp Ala
    130
```

(2) INFORMATION FOR SEQ ID NO:3:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 324 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
GATATCCAGA TGACACAGAC TACATCCTCC CTGTCTGCCT CTCTGGGAGA CAGAGTCACC      60

ATCAGTTGCA GGGCAAGTCA GGACATTAGC AATTATTTAA ACTGGTATCA GCAGAAACCA     120

GATGGAACTG TTAAACTCCT GATCTACTAC ACATCAAGAT TACACTCAGG AGTCCCATCA     180

AGGTTCAGTG GCAGTGGGTC TGGAACAGAT TATTCTCTCA CCATTAGCAA CCTGGAGCAA     240

GAAGATATTG CCACTTACTT TTGCCAACAG GGTAATACGC TTCCTCCGAC GTTCGGTGGA     300

GGCACCAAGC TGGAAATCAA ACGG                                           324
```

(2) INFORMATION FOR SEQ ID NO:4:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 285 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
GATATCCAGA TGACACAGAC TACATCCTCC CTGTCTGCCT CTCTGGGAGA CAGAGTCACC      60

ATCAGTTGCA GGGCAAGTCA GGACATTAGC AATTATTTAA ACTGGTATCA GCAGAAACCA     120

GATGGAACTG TTAAACTCCT GATCTACTAC ACATCAAGAT TACACTCAGG AGTCCCATCA     180

AGGTTCAGTG GCAGTGGGTC TGGAACAGAT TATTCTCTCA CCATTAGCAA CCTGGAGCAA     240

GAAGATATTG CCACTTACTT TTGCCAACAG GGTAATACGC TTCCT                     285
```

-38-

.(2) INFORMATION FOR SEQ ID NO:5:

    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 39 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: double
      (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TGGACGTTCG GTGGAGGCAC CAAGCTGGAA ATCAAACGT   39

(2) INFORMATION FOR SEQ ID NO:6:

    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 429 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: double
      (D) TOPOLOGY: linear

    (ix) FEATURE:
      (A) NAME/KEY: CDS
      (B) LOCATION: 1..429

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
ATG GGA TGG AGC TGT ATC ATC CTC TTT TTG GTA GCA GCA GCT ACA GGT          48
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Ala Ala Thr Gly
 1               5                  10                  15

GTC CAC TCC CAG GTC CAA CTG CAG CAG CCT GGG ACT GAA CTG GTG AAG.         96
Val His Ser Gln Val Gln Leu Gln Gln Pro Gly Thr Glu Leu Val Lys
                 20                  25                  30

CCT GGG GCT TCA GTG AAG CTG TCC TGC AAG GCT TCT GGC TAC ACC TTC        144
Pro Gly Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe
                 35                  40                  45

ACC AGC TAC TGG ATG CAC TGG GTG AAG CAG AGG CCT GGA CAA GGC CTT        192
Thr Ser Tyr Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
                 50                  55                  60

GAG TGG ATT GGA AAT ATT AAT CCT AGC AAT GGT GGT ACT AAC TAC AAT        240
Glu Trp Ile Gly Asn Ile Asn Pro Ser Asn Gly Gly Thr Asn Tyr Asn
 65                  70                  75                  80

GAG AAG TTC AAG AGC AAG GCC ACA CTG ACT GTA GAC AAA TCC TCC AGC        288
Glu Lys Phe Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser
                 85                  90                  95

ACA GCC TAC ATG CAG CTC AGC AGC CTG ACA TCT GAG GAC TCT GCG GTC        336
Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
                 100                 105                 110

TAT TAT TAT GCA AGA CGG GCC CCT TAC TAC GGT AGT AGG AAC TTT GAC        384
Tyr Tyr Tyr Ala Arg Arg Ala Pro Tyr Tyr Gly Ser Arg Asn Phe Asp
                 115                 120                 125

TAC TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA GAG AGT CAG           429
Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Glu Ser Gln
                 130                 135                 140
```

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(2) INFORMATION FOR SEQ ID NO:7:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 143 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Ala Ala Thr Gly
 1               5               10              15

Val His Ser Gln Val Gln Leu Gln Gln Pro Gly Thr Glu Leu Val Lys
            20              25              30

Pro Gly Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe
        35              40              45

Thr Ser Tyr Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
    50              55              60

Glu Trp Ile Gly Asn Ile Asn Pro Ser Asn Gly Gly Thr Asn Tyr Asn
65              70              75              80

Glu Lys Phe Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser
            85              90              95

Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
            100             105             110

Tyr Tyr Tyr Ala Arg Arg Ala Pro Tyr Tyr Gly Ser Arg Asn Phe Asp
        115             120             125

Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Glu Ser Gln
    130             135             140
```

(2) INFORMATION FOR SEQ ID NO:8:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 366 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (ix) FEATURE:
        (A) NAME/KEY: CDS
        (B) LOCATION: 1..366

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
CAG GTC CAA CTG CAG CAG CCT GGG ACT GAA CTG GTG AAG CCT GGG GCT    48
Gln Val Gln Leu Gln Gln Pro Gly Thr Glu Leu Val Lys Pro Gly Ala
 1               5               10              15

TCA GTG AAG CTG TCC TGC AAG GCT TCT GGC TAC ACC TTC ACC AGC TAC    96
Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20              25              30
```

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```
TGG ATG CAC TGG GTG AAG CAG AGG CCT GGA CAA GGC CTT GAG TGG ATT          144
Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
        35              40                  45

GGA AAT ATT AAT CCT AGC AAT GGT GGT ACT AAC TAC AAT GAG AAG TTC          192
Gly Asn Ile Asn Pro Ser Asn Gly Gly Thr Asn Tyr Asn Glu Lys Phe
    50              55                  60

AAG AGC AAG GCC ACA CTG ACT GTA GAC AAA TCC TCC AGC ACA GCC TAC          240
Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65              70                  75                  80

ATG CAG CTC AGC AGC CTG ACA TCT GAG GAC TCT GCG GTC TAT TAT TAT          288
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Tyr
                85                  90                  95


GCA AGA GAT TAC TAC GGT AGT AGC TGG GGG TAC TAC TTT GAC TAC TGG          336
Ala Arg Asp Tyr Tyr Gly Ser Ser Trp Gly Tyr Tyr Phe Asp Tyr Trp
            100                 105                 110

GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA                                  366
Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
            115                 120
```

(2) INFORMATION FOR SEQ ID NO:9:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 122 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Gln Val Gln Leu Gln Gln Pro Gly Thr Glu Leu Val Lys Pro Gly Ala
 1               5               10                  15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20              25                  30

Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
        35              40                  45

Gly Asn Ile Asn Pro Ser Asn Gly Gly Thr Asn Tyr Asn Glu Lys Phe
    50              55                  60

Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65              70                  75                  80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Tyr
                85                  90                  95

Ala Arg Asp Tyr Tyr Gly Ser Ser Trp Gly Tyr Tyr Phe Asp Tyr Trp
            100                 105                 110

Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
            115                 120
```

(2) INFORMATION FOR SEQ ID NO:10:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 351 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
ATGGGATGGA GCTGTATCAT CCTCTTTTTG GTAGCAGCAG CTACAGGTGT CCACTCCCAG        60

GTCCAACTGC AGCAGCCTGG GACTGAACTG GTGAAGCCTG GGGCTTCAGT GAAGCTGTCC       120

TGCAAGGCTT CTGGCTACAC CTTCACCAGC TACTGGATGC ACTGGGTGAA GCAGAGGCCT       180

GGACAAGGCC TTGAGTGGAT TGGAAATATT AATCCTAGCA ATGGTGGTAC TAACTACAAT       240

GAGAAGTTCA AGAGCAAGGC CACACTGACT GTAGACAAAT CCTCCAGCAC AGCCTACATG       300

CAGCTCAGCA GCCTGACATC TGAGGACTCT GCGGTCTATT ATTATGCAAG A               351
```


(2) INFORMATION FOR SEQ ID NO:11:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 45 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
TACTTTGACT ACTGGGGCCA AGGCACCACT CTCACAGTCT CCTCA                        45
```

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CLAIMS

What is claimed is:

1. A method of stimulating remyelination of central
    nervous system axons in a mammal comprising
5   administering to the mammal an effective amount of a
    monoclonal antibody produced by the hybridoma having
    the ATCC Accession No. CRL 11627.

2. The method of Claim 1 wherein the method of
    administration is intravenous administration.

10 3. The method of Claim 1 wherein the method of
    administration is intraperitoneal administration.

4. The method of Claim 1 wherein said amount of monoclonal
    antibody administered is between from about 0.5 mg/kg
    to about 400 mg/kg.

15 5. A method of stimulating the proliferation of glial
    cells in central nervous system axons in a mammal
    comprising administering to the mammal an effective
    amount of a monoclonal antibody produced by the
    hybridoma having the ATCC Accession No. CRL 11627.

20 6. The method of Claim 5 wherein the method of
    administration is intravenous administration.

7. The method of Claim 5 wherein the method of
    administration is intraperitoneal administration.

8. The method of Claim 5 wherein said amount of monoclonal
antibody administered is between from about 0.5 mg/kg
to about 400 mg/kg.

9. A method of treating a demyelinating disease of the
5   central nervous system in a mammal comprising
administering to said mammal an effective amount of a
monoclonal antibody produced by the hybridoma having
the ATCC Accession No. CRL 11627 to promote
remyelination of central nervous system axons of said
10 mammal.

10. The method of Claim 9 wherein said mammal is a human
being having multiple sclerosis, or a human or domestic
animal with a viral demyelinating disease, or a post-
neural disease of the central nervous system.

15 11. The method of Claim 9 wherein the method of
administration is intravenous administration.

12. The method of Claim 9 wherein the method of
administration is intraperitoneal administration.

13. The method of Claim 9 wherein said amount of monoclonal
20 antibody administered is between from about 0.5 mg/kg
to about 400 mg/kg.

14. The method of Claim 9 wherein said mammal is a mouse
infected with Strain DA of Theiler's murine
encephalomyelitis virus.

25 15. A hybridoma comprising hybridoma ATCC accession No. CRL
11627 that produces a monoclonal antibody capable of
stimulating remyelination of central nervous system
axons.

16. An _in vitro_ method of producing glial cells comprising:

    a)   culturing glial cells under conditions sufficient for cell proliferation, thereby producing a glial cell culture;

5   b)   introducing into the glial cell culture an effective amount of a monoclonal antibody produced by the hybridoma having the ATCC Accession No. CRL 11627, thereby producing a monoclonal antibody-treated glial cell culture;

10   c)   maintaining the culture of step b) under conditions sufficient for proliferation of monoclonal antibody-treated cells; and

    d)   harvesting the cells from culture, thereby obtaining glial cells.

15 17. Glial cells obtained by the method of Claim 16.

18. An _in vitro_ method of stimulating the proliferation of glial cells from mixed cell culture comprising:

    a)   culturing a mixed cell culture containing glial cells under conditions sufficient for cell

20   proliferation;

    b)   introducing into the mixed culture an effective amount of a monoclonal antibody produced by the hybridoma having the ATCC Accession No. CRL 11627, thereby producing a monoclonal antibody-treated

25   mixed culture;

    c)   maintaining the culture of step b) under conditions sufficient for proliferation of monoclonal antibody-treated cells, thereby resulting in the proliferation of glial cells in

30   the mixed culture; and

    d)   harvesting the glial cells from the mixed culture.

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19. The method of Claim 18 wherein the mixed culture is
     obtained from rat optic nerve.

20. The method of Claim 18 wherein the mixed culture is
     obtained from rat brain.

5 21. Glial cells obtained by the method of Claim 18.

22. A method of stimulating remyelination of central
     nervous system axons in a mammal comprising;
     a)   culturing glial cells under conditions sufficient
          for cell proliferation, thereby producing a glial
10   cell culture;
     b)   introducing into the glial cell culture an
          effective amount of a monoclonal antibody produced
          by the hybridoma having the ATCC Accession No. CRL
          11627, thereby producing a monoclonal antibody-
15   treated glial cell culture;
     c)   maintaining the culture of step b) under
          conditions sufficient for proliferation of
          monoclonal antibody-treated cells;
     d)   harvesting the monoclonal antibody-treated cells
20   from culture, thereby obtaining glial cells; and
     e)   introducing the glial cells obtained in step d)
          into the central nervous system of the mammal,
          thereby stimulating remyelination of central
          nervous system axons.

25 23. A monoclonal antibody producible from hybridoma
     according to Claim 15.

24. A monoclonal antibody according to Claim 23 for use in
     therapy.

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25. Use of monoclonal antibody according to Claim 23 for
     the manufacture of a medicament for stimulating
     remyelination of central nervous system axons.

26. Use of a monoclonal antibody according to Claim 23 for
 5   the manufacture of a medicament for stimulating the
     proliferation of glial cells in central nervous system
     axons.

27. Use of a monoclonal antibody according to Claim 23 for
     the manufacture of a medicament for treating a
10   demyelinating disease of the central nervous system
     e.g. multiple sclerosis, a viral demyelinating disease,
     or a post-neural disease of the central nervous system.

28. Use according to Claim 27, wherein the medicament is
     for treating a mouse infected with Strain DA of
15   Theiler's murine encephalomyelitis virus.

29. Use according to any one of Claims 25, 26 and 27,
     wherein the method of administration is intravenous
     administration, or intraperitoneal administration.

30. Use according to any one of Claims 25 to 28, wherein
20   said amount of monoclonal antibody administered is from
     about 0.5 mg/kg to about 400 mg/kg.

31. A method of preparing glial cells for stimulating
     remyelination of central nervous system axons in a
     mammal comprising;
25   a)   culturing glial cells under conditions sufficient
          for cell proliferation, thereby producing a glial
          cell culture;
     b)   introducing into the glial cell culture an
          effective amount of a monoclonal antibody produced

by the hybridoma having the ATCC Accession No. CRL
11627, thereby producing a monoclonal antibody-
treated glial cell culture;

    c)   maintaining the culture of step b) under
5   conditions sufficient for proliferation of
monoclonal antibody-treated cells from culture,
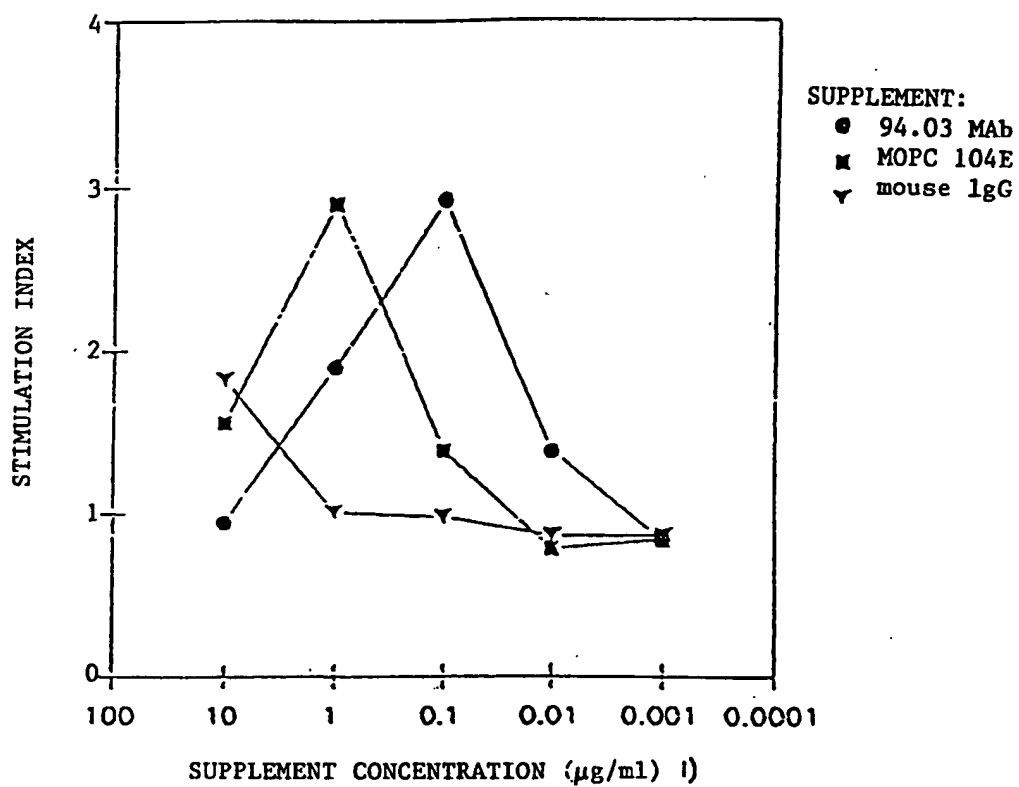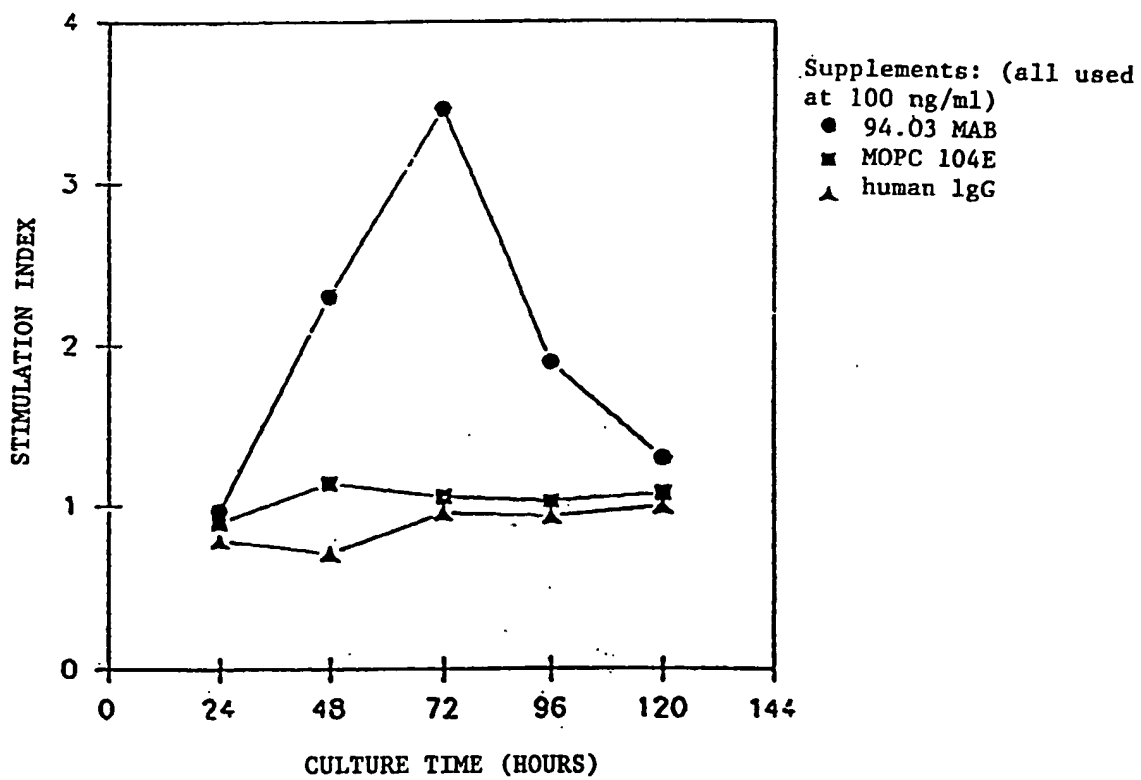thereby obtaining glial cells.

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Figure 1

Figure 2

FIG. 3A
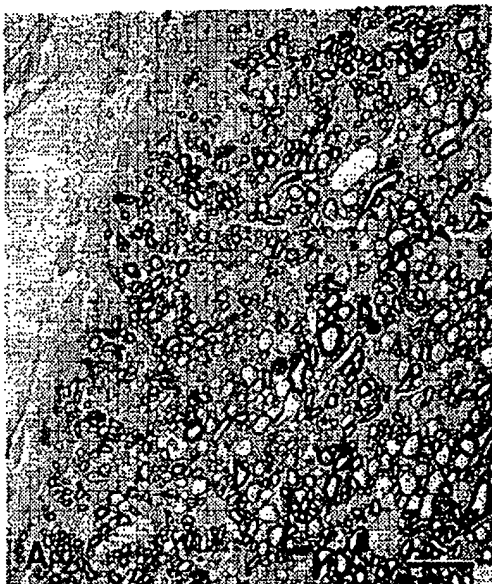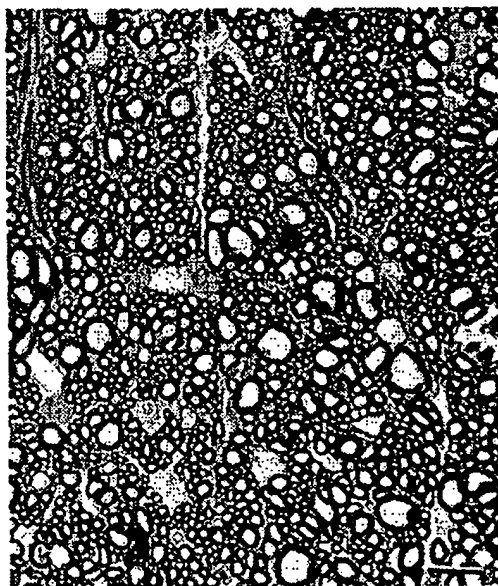
FIG. 3B

FIG. 3C

FIG. 3D

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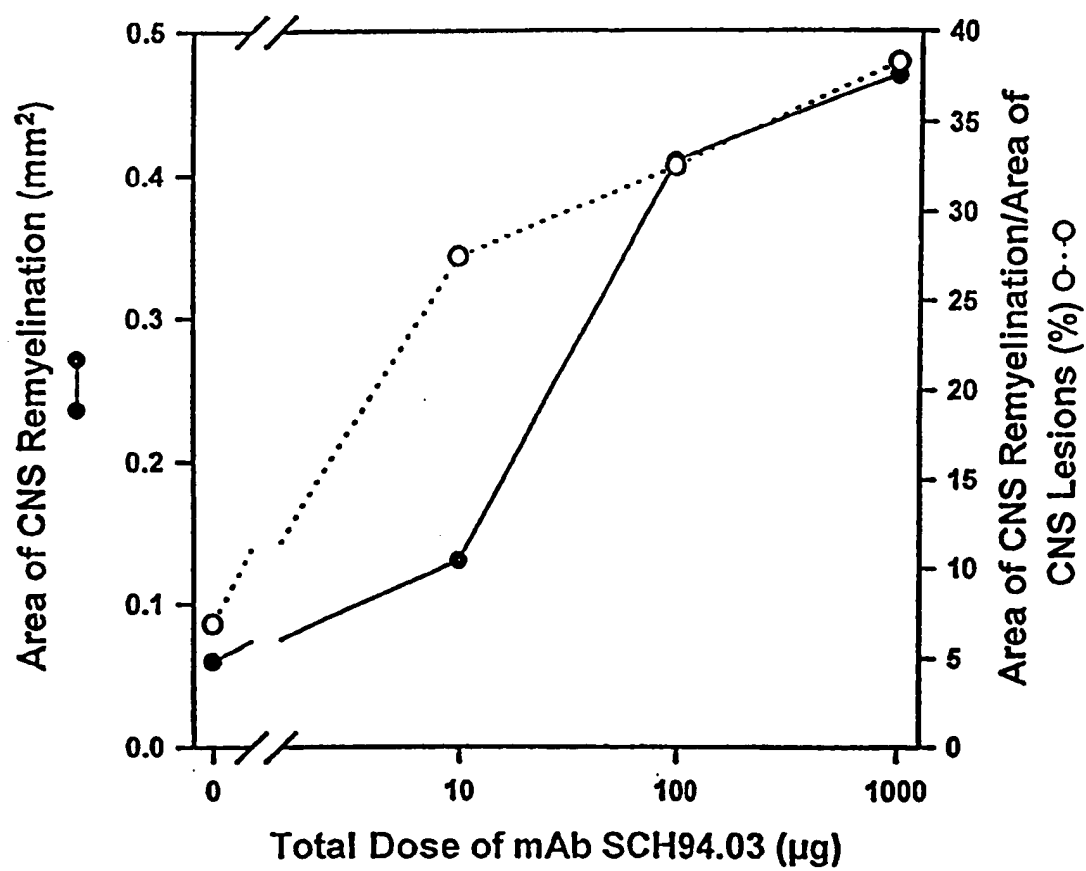
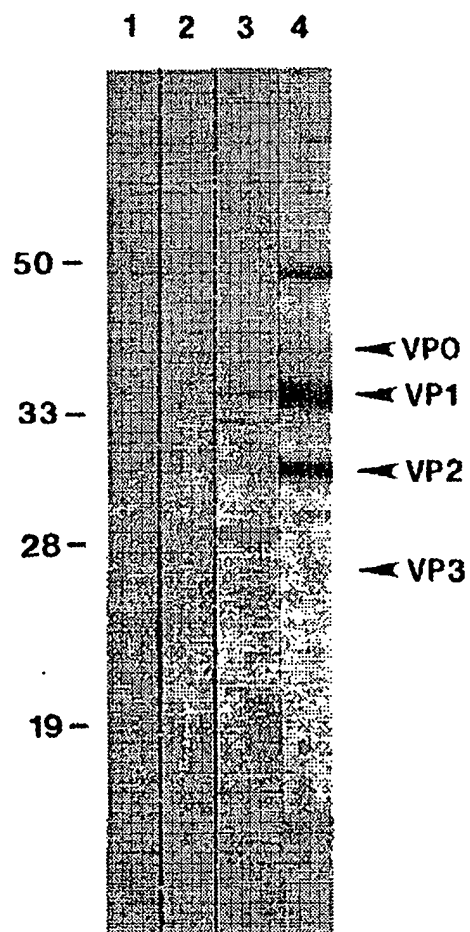
Figure 4

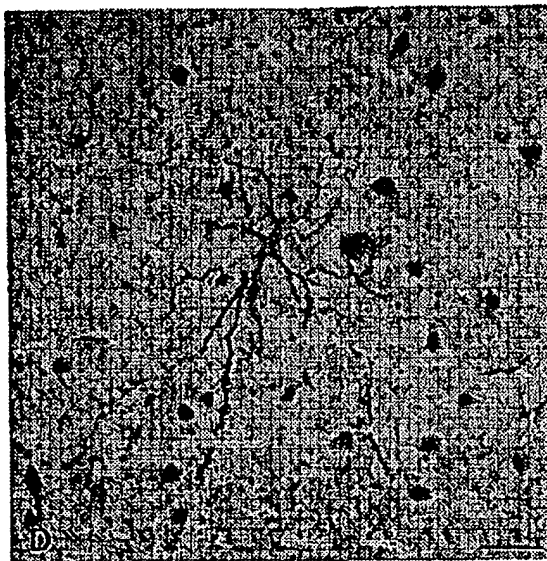Figure 5

FIG. 6

FIG. 7A   FIG. 7B

FIG. 7C   FIG. 7D

Fig. 8A

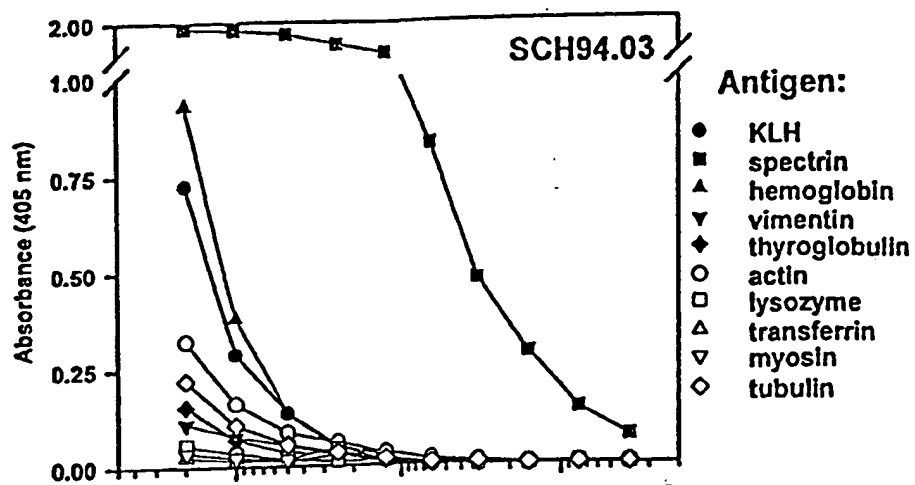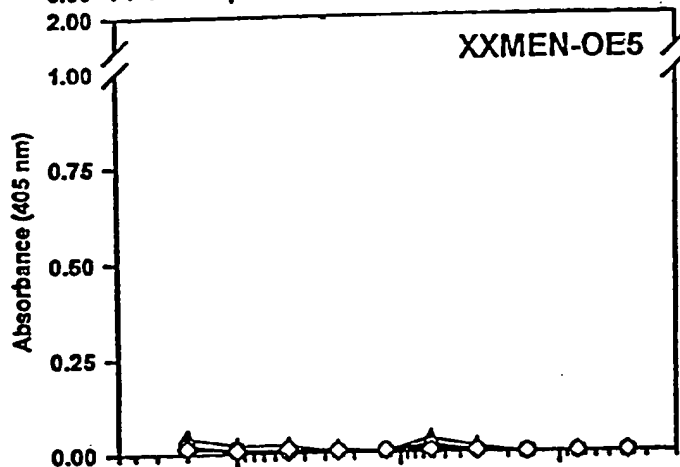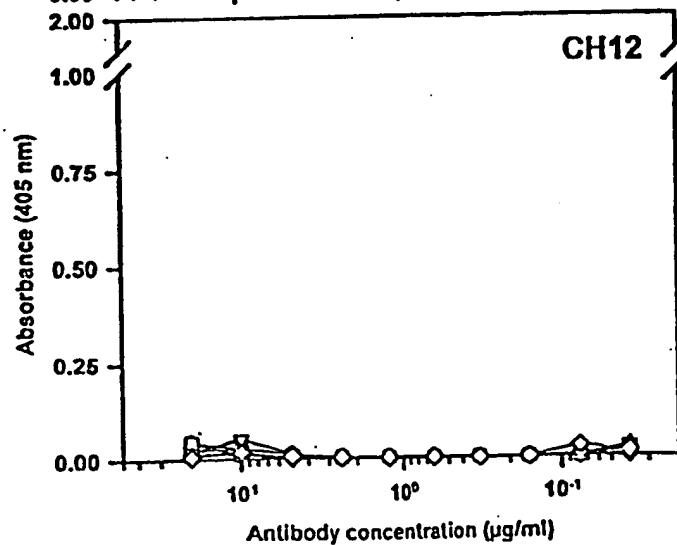Fig. 8B

Fig. 8C

Figure 9

Fig. 10A

Fig. 10B

Fig. 10C

Fig. 11A

Leader region

```
          -19  M   M   S   S   A   Q   F   L   G   L   L   L   C   F   Q   G   T   R   C   D   I   Q   M   T   Q   T
SCH94.03       ATG ATG TCC TCT GCT CAG TTC CTT GGT CTC CTG TTG CTC TGT TTT CAA GGT ACC AGA TGT GAT ATC CAG ATG ACA CAG ACT
CH12                                                                                                          --- --- ---
germline Vk12                                                                                                 --- --- ---
```

CDR1

```
          10  T   S   L   S   A   S   L   G   D   R   V   T   I   S   C   R   A   S   Q   D   I   S   N   Y   L   N
SCH94.03       ACA TCC TCC CTG TCT GCC TCT CTG GGA GAC AGA GTC ACC ATC AGT TGC AGG GCA AGT CAG GAC ATT AGC AAT TAT TTA AAC
CH12           ---                                                                                            --- --- ---
germline Vk12  ---                                                                                            --- --- ---
```

CDR2

```
          40  W   Y   Q   Q   K   P   D   G   T   V   K   L   L   I   Y   Y   T   S   R   L   H   S   G   V   P   R
SCH94.03       TGG TAT CAG CAG AAA CCA GAT GGA ACT GTT AAA CTC CTG ATC TAC TAC ACA TCA AGA TTA CAC TCA GGA GTC CCA TCA AGG
CH12                                                                                                          --- --- ---
germline Vk12                                                                                                 --- --- ---
```

```
          70  F   S   G   S   G   S   G   T   D   Y   S   L   T   I   S   N   L   E   Q   E   D   I   A   T   Y   C
SCH94.03       TTC AGT GGC AGT GGG TCT GGG ACA GAT TAT TCT CTC ACC ATT AGC AAC CTG GAG CAA GAA GAT ATT GCC ACT TAC TTT TGC
CH12                                                                                                          --- --- ---
germline Vk12                                                                                                 --- --- ---
```

CDR3

```
          90  Q   Q   G   N   T   L   P   W   T   F   G   G   G   T   K   L   E   I   K   R   A   D   A
SCH94.03       CAA CAG GGT AAT ACG CTT CCG TGG ACG TTC GGT GGA GGC ACC AAG CTG GAA ATC AAA CGG GCT GAT GCT
CH12                           --- --- CC-     ---                                                 ---
germline Vk12                  --T --- --T     ---                                                 ---     --T
Jk1                            --T                                                                 ---     --T
```
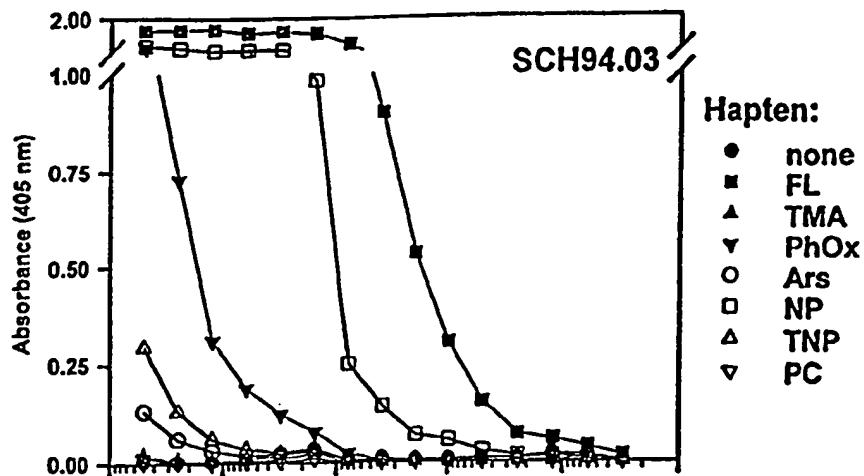
J region

Cĸ

Figure 11B
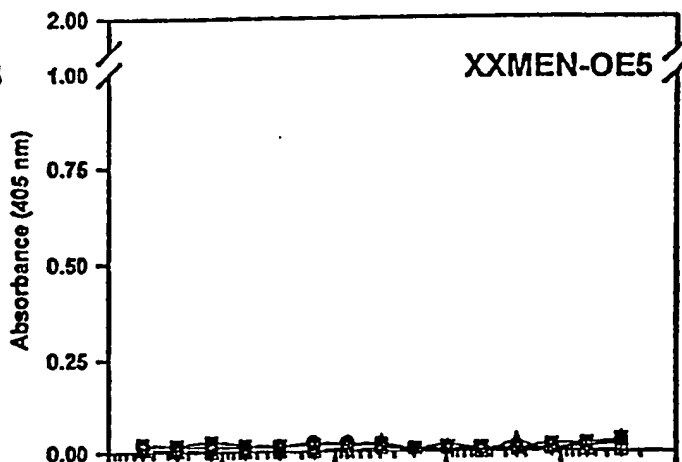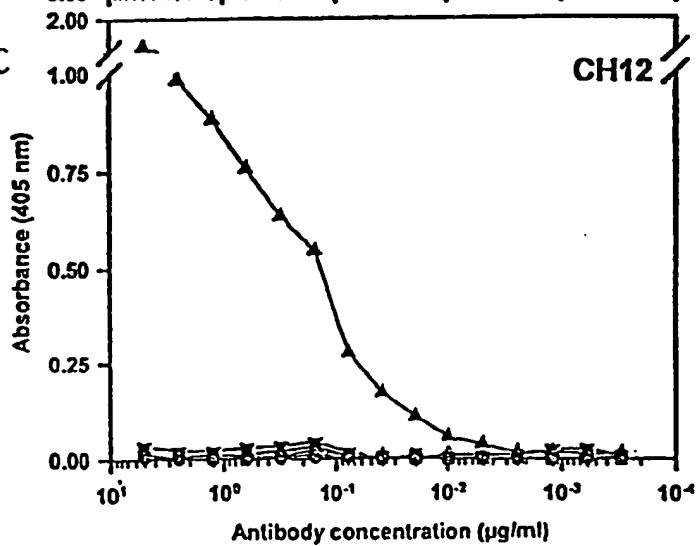
INTERNATIONAL SEARCH REPORT

Internatic˙ ˙ Application No

PCT/U⌐ 95/05262

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   C12N15/13   A61K39/395   C07K16/18   C12N5/20   C12N5/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6   C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE MEDLINE FILE SERVER STN KARLSRUHE ABSTRACT NO.91374165, PATICK ET AL 'PERSISTENCE OF THEILER'S VIRUS INFECTION FOLLOWING PROMOTION OF CENTRAL NERVOUS SYSTEM REMYELINATION' cited in the application	23-30
A	& J NEUROPATHOL EXP NEUROL, (1991 SEP) 50 (5) 523-37 see abstract --- -/--	1-22,31

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 August 1995	2 8. 08. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Sitch, W

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page 1 of 3

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE MEDLINE FILE SERVER STN KARLSRUHE ABSTRACT NO.90146187, RODRIGUEZ ET AL 'IMMUNOGLOBULINS PROMOTE REMYELINATION IN THE CENTRAL NERVOUS SYSTEM '	23-30
A	& ANN NEUROL, (1990 JAN) 27 (1) 12-7 see abstract ---	1-22,31
X	DATABASE MEDLINE FILE SERVER STN KARLSRUHE ABSTRACT NO.87085707, RODRIGUEZ ET AL 'REMYELINATION BY OLIGODENDROCYTES STIMULATED BY ANTISERUM TO SPINAL CORD'	23-30
A	& J NEUROPATHOL EXP NEUROL, (1987 JAN) 46 (1) 84-95 see abstract ---	1-22,31
X	DATABASE MEDLINE FILE SERVER STN KARLSRUHE ABSTRACT NO.91163050, RODRIGUEZ 'IMMUNOGLOBULINS STIMULATE CENTRAL NERVOUS SYSTEM REMYELINATION:ELECTRON MICROSCOPIC AND MORPHOMETRIC ANALYSIS OF PROLIFERATING CELLS'	23-30
A	& LAB INVEST, (1991 MAR) 64 (3) 358-70 see abstract ---	1-22,31
X	WO-A-92 04442 (UNIV CALIFORNIA) 19 March 1992 see page 19, line 6 - page 24, line 14 ---	23-30
X	NEUROLOGY, vol. 42, no. 2, February 1992 pages 348-357, RODRIGUEZ ET AL 'IMMUNOSUPPRESSION PROMOTES CNS REMYELINATION IN CHRONIC VIRUS-INDUCED DEMYELINATING DISEASE' cited in the application see the whole document ---	23-30
P,X	THE JOURNAL OF IMMUNOLOGY, vol. 154, no. 5, 1 March 1995 pages 2460-2469, MILLER ET AL 'A MONOCLONAL AUTOANTIBODY THAT PROMOTES CENTRAL NERVOUS SYSTEM REMYELINATION IN A MODEL OF MULTIPLE SCLEROSIS IS A NATURAL AUTOANTIBODY ENCODED BY GERMLINE IMMUNOGLOBULIN GENES' see the whole document ---	1-31

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1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	THE JOURNAL OF NEUROSCIENCE, vol. 14, no. 10, October 1994 pages 6230-6238, MILLER ET AL 'MONOCLONAL AUTOANTIBODIES PROMOTE CENTRAL NERVOUS SYSTEM REPAIR IN AN ANIMAL MODEL OF MULTIPLE SCLEROSIS' see the whole document ---	1-31
P,X	PROGRESS IN BRAIN RESEARCH, vol. 103, 23 December 1994 pages 343-355, RODRIGUEZ ET AL 'IMMUNE PROMOTION OF CENTRAL NERVOUS SYSTEM REMYELINATION' see the whole document -----	1-31

Box I   Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:   **1-4,22**
   because they relate to subject matter not required to be searched by this Authority, namely:
   Remark: Although claims 1-14,22 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9204442	19-03-92	AU-A- 8864691	30-03-92